Fluoride Uptake by Enamel From Stannous Fluoride and Prophylaxis Pastes

By

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INTRODUCTION

Investigators have noted the marked prevalence of dental caries in children as young as 18 months of age. Hennon, Stookey, and Muhler[1], in a 1969 survey of 915 children, found an 8.3 per cent dental caries prevalence in the 18- to 23-month-old subjects. This factor increased to over 57 per cent in those who were 36 to 39 months of age. In this latter subgroup, there was a mean decayed, extracted, or filled (def) surface value of 6.16. Similar findings had been noted by Wisan, Lavell, and Colwell[2] in 1957. They found the prevalence of dental caries to be 22, 52, and 74 per cent in children aged two, three, and five years, respectively. The overall prevalence level for these 2677 children was 60 per cent, with a skewing toward those of lower income families. Toverud and others[3] wrote of similarly disheartening findings in first permanent molars. Ninety per cent of the 12-year-olds in their survey had dental caries or a history of decay in one or more such teeth. Perhaps even more indicative of the prevalence of dental caries in children is the investigators' statement that fully 20 per cent of six year olds had decay in permanent molars.

The fact that this disease is so widespread may have influenced the general public to consider its sequelae (restorations and extractions) to be among the normal milestones in growing up and aging. Both McDonald[4] and Volker and Russell[5] stated that more than four billion dollars was spent in 1970 (presumably in the United States alone) for dental care, and that the vast preponderance of this sum reflected restorations of carious teeth. This fatalistic approach has made the prevention of dental caries a most formidable task.

Three broad avenues of attack are considered potentially effective in caries prevention: removing cariogenic microorganisms, reducing the numbers of substrates which permit the growth and development of colonies of these microorganisms, and increasing the resistance on a microscopic level of the dental hard structures to the acids elaborated by these colonial microorganisms called plaque. No specific technique has been shown to be totally effective clinically. Therefore although this study will dwell on one small portion of the last of these three approaches, it is understood that the highest level of dental caries reduction is achieved ideally through a combination of approaches.

As early as the first quarter of this century, empirical observations were completed of community-wide intrinsic dental staining coupled with a greatly reduced dental caries rate[6,7]. This stain was later recognized as dental fluorosis, induced by an unusually high level of the fluoride ion in the communal water supply[8,9]. Gradually, observers realized that in lesser amounts, the fluoride ion might still impart some type of caries-resistant quality to dental enamel without the drawback of the brown, mottled stain[10,11]. Various vehicles for topical and systemic administration of this ion were suggested, then explored in the laboratory and in field studies. Besides its natural presence in drinking water, fluoride could be added to the communal water supply, it could be either rinsed or applied periodically in higher concentrations directly in the mouth, it could be incorporated into various cleaning and polishing pastes, and it could be given as a dietary supplement.

In this thesis two commonly accepted modalities of fluoride therapy will be reviewed: periodic application of a 10 per cent aqueous solution of

of stannous fluoride and periodic application of nine per cent stannous fluoride as a component of a zirconium silicate cleaning and polishing paste. As a practical matter, many dentists combine these two techniques in their preventive programs. The present study sought to determine whether the combination of these two approaches provides an additive effect in fluoride uptake. A short-term clinical investigation was conducted which used a modification of an *in vivo* enamel biopsy technique by Hotz[12] and his co-workers.

The following hypotheses were tested:

1. When a zirconium silicate prophylaxis paste is applied prior to topical application of 10 per cent aqueous solution of stannous fluoride, the presence of nine per cent stannous fluoride in the paste does not promote a significantly greater enamel uptake of fluoride than when no stannous fluoride is added to the zirconium silicate prophylaxis paste.

2. Rinsing the mouth with tap water immediately after application of zirconium silicate prophylaxis paste (containing nine per cent stannous fluoride) followed topical application of 10 per cent aqueous solution of stannous fluoride does not significantly affect enamel fluoride uptake.

REVIEW OF THE LITERATURE

I. In Vitro Topical Fluoride Studies

Early observations of the effects of fluoride on tooth enamel[6-9] were based on inferential conclusions that people using water supplies containing high concentrations of fluoride also had special and similar dental findings. However, proof of a causal relationship between fluoride levels and altered physical properties of dental enamel awaited laboratory examination. An attempt to demonstrate this was first reported by Volker[11] in 1939. He treated powdered enamel with varying aqueous dilutions of sodium fluoride (ranging from 0.01 per cent to as high as four per cent) for one hour periods. He then measured the dissolution rate of these samples with exposure for one hour in 0.2M acetic acid, buffered to a pH of 4.0. He noted a reduced degree of enamel solubility, regardless of fluoride concentrations. Untreated samples lost over 54 per cent weight with this decalcification procedure, while weight losses of 26 per cent to 37 per cent were measured in the classes of treated sample.

With this first indication of causality, an increasing number of researchers examined the effects of fluoride upon enamel. The authors in the next 13 citations expanded upon Volker's[11] work. They provided *in vitro* demonstrations of the effects of various fluoride agents on the physical properties of dental enamel.

Muhler and Van Huysen[14] in 1947 measured the dissolution rate of powdered tooth enamel in the presence of acetic acid as a function of pretreatment with one of 27 compounds. Stannous fluoride was the most effective.

The same authors[15] later focused specifically on a comparison of sodium fluoride solutions of different concentrations (0.2 to 4.0 per cent), both buffered and unbuffered. Again using the dissolution rate of powdered tooth enamel in the presence of acetic acid as the mechanism for measurement, they found a decreased enamel solubility rate with two per cent solutions of sodium fluoride at a pH of 4.5.

Manley and Bibby[16] in 1949 reported on an examination of 147 substances composed of one or more of 57 different elements. Aqueous or buffered acetic acid solutions were made. Again, powdered tooth enamel solubility rates were measured. Sodium fluoride was reported to be less active than 27 other substances. However, stannous fluoride was not among the 147 test substances.

Ericsson[17] turned his attention in 1950 to solubility of whole tooth sections. He rubbed his test solutions onto extracted cuspids and biscuspids, using cotton wool. The teeth had been previously sectioned lengthwise. Half of each tooth served as the control for the other half. A total of 13 agents were examined, including five per cent sodium fluoride, 0.1 per cent sodium fluoride, and stannous fluoride with a fluoride ion content corresponding to two per cent sodium fluoride. Each

sample was exposed to 0.001N hydrochloric acid for two hours; decalcification products were then measured. Stannous fluoride appeared superior to all other agents in the reduction of dissolution rates.

Brewer, Muhler, and Fischer[18] in 1956 examined stannous fluoride and sodium fluoride in terms of changes in enamel permeability. This variable was evaluted using techniques of chemical microscopy and electrical conductance. With the first technique, two per cent solutions of stannous fluoride reduced enamel permeability, while one percent solutions of sodium fluoride had no such effect. When measured via electrical conductance, both agents reduced enamel permeability, but the effect was more than twice as marked after treatment with stannous fluoride than after sodium fluoride.

Gray and his co-workers,[19] also in 1956, examined dental enamel by electron microscopy following acid application. Enamel which had previously been treated with stannous fluoride fared better, in their opinion, than enamel that had been treated with sodium fluoride. The latter agent further eroded the surface enamel, depositing crystals thought to be calcium fluoride.

In the same year, Radike and his associates[20] considered a new approach for their research. They treated teeth with either stannous fluoride or sodium fluoride, using solutions ranging in pH from 2.5 to 5.5. Their test teeth had previously been made radioactive and were now mounted in plastic with only the crowns exposed. The radioactivity of the acid decalcification solutions was measured. Regardless of test agent pH, tagged ions were twice as numerous in the solutions from teeth treated with

stannous fluoride, than in those from teeth treated with sodium fluoride. The authors felt that this test corroborated Muhler's[14,15] *in vitro* examination of powdered dental enamel, in that more stannous fluoride than sodium fluoride had been taken up. However, the results may have reflected instead a higher solubility effect upon enamel treated with stannous fluoride than sodium fluoride.

Hals[21] in 1957 reported on a study in which experimental teeth were prepared in a method similar to that of Ericsson[17]. After treatment, the teeth were incubated in a 10 per cent sucrose in saliva solution. Resistance to acid dissolution was then measured by examining grouped sections in polarized light. The results corroborated those of Ericsson.

Also in 1957, Mühlemann[22] reported on the protection afforded by four to 20 hour treatments of dental enamel with a number of inorganic compounds. A series of decalcification stages over a three-hour period made it possible to determine the decalcification rate. Of those agents used, stannous fluoride afforded the greatest protection from subsequent acid decalcification. At one hour, teeth treated with it were three to five times as resistant to decalcification as were teeth treated with sodium fluoride. At three hours, this advantage had been reduced to a 50 per cent difference. Fifty per cent aqueous phthalate buffer, with a pH of 4, was the decalcification agent.

Muhler[23] in 1957, recognizing variability in acid dissolution among different teeth and from area to area within a given tooth, devised a method of repeatedly decalcifying the same area of a tooth. Teeth were imbedded in Wood's metal and exposed to four 15 minute periods of 0.2M

acetic acid (with an adjusted pH of 4.0). Then the surface was treated for 20 minutes with one of 10 agents (eight of which contained fluoride); stannous fluoride and sodium fluoride were among these. The teeth were then subjected to additional decalcifications. The last pretreatment decalcification value (based on the amount of phosphorus lost) was compared with the first post-treatment value. The difference, termed "per cent protection" by the author, was greatest among the 10 agents for stannous fluoride. However, as will be later demonstrated, different depths in the same area have different mineral constituents, and thus consecutive decalcifications can provide misleading values.

Also in 1957, Scott, Hernandez, and McConnell[24] reported on an electron microscopy study in which teeth were sectioned longitudinally and half of each was treated with 0.4 per cent stannous fluoride or 0.2 per cent sodium fluoride. Following five-minute decalcifications in 0.1N lactic or hydrochloric acid, the teeth were examined. The researchers' conclusions paralleled those of the other authors who had used this technique, namely that stannous fluoride provided a more marked resistance to etching than did sodium fluoride.

Walsh and his fellow investigators[25] performed repeated decalcifications on treated powdered enamel. Reporting in 1957, they concluded that stannous fluoride was more effective than sodium fluoride in reducing rates of enamel dissolution, particularly when the agents were at lower pH values.

Ericsson[26] used a technique of radioactivity in 1961. However, unlike Radike and his associates[20], Ericsson used F^{18}-labelled ions, rather

than bombarding the untreated teeth with radioactivity. Experimentation was completed with both intact and powdered enamel. He agreed that stannous fluoride uptake exceeded that of sodium fluoride, especially at pH of 4.1.

II. In Vivo Topical Fluoride Studies

In a series of five papers published in the early 1950's Muhler and co-workers[27-31] described the clinical effect upon dental caries of dietary fluoride agents in the food and water of rats and hamsters. Both stannous fluoride and sodium fluoride reduced the incidence of dental caries when the animals were fed a caries-producing diet. Stannous fluoride-induced reductions were about double those of sodium fluoride.

Bibby[32,33] in 1942 and 1944 reported on the first clinical studies using a topical fluoride agent. Using 90 subjects from a non-fluoride area who were 10 to 13 years of age, he applied 0.1 per cent sodium fluoride for seven to eight minutes every four months. One and two year decayed, missing, or filled (DMF) surface reductions were 30.9 and 27.6 per cent, respectively.

Knutson and Armstrong[34-36] reported between 1943 and 1946 on a similar study on a larger scale. They began with 337 participants (also in an area of low communal fluoride levels) ranging in age from 7 to 15 years. Two per cent sodium fluoride was applied for three minutes twice weekly for three to eight weeks. The rate of DMF teeth was reduced by 39.7 per cent after one year, 41.4 per cent after two, and 36.7 per

cent after three years. Corresponding DMF surface reductions were measured as 23.4, 34.6, and 32.8 per cent.

Both Bibby and Knutson relied on the half-mouth technique of experimental design, whereby each subject serves as his own control. Implicit in this design is the statement that any given agent applied to one side of the mouth can indeed be contained to that side of the mouth. In 1964 Meckel and Francis[37] demonstrated that, on the contrary, these techniques were not experimentally valid; the active ions were measured on the untreated side. In a similar vein, Buttner and Muhler[38] in 1957 reported difficulty in confining topical agents to the mandibular arches of laboratory animals.

While the above five citations refer to studies on children, the following seven references are based on examination of adults using sodium fluoride topical applications. As with the studies on children, these studies were confined to areas having inadequate communal fluoride levels.

Arnold, Dean, and Singleton[39] in 1944 offered one year results on 94 subjects aged 17 to 23 years. They had received one five-minute application of one per cent sodium fluoride. There was no significant dental caries reduction in this whole-mouth experiment.

Klinkenberg and Bibby[40] six years later reported a 47.3 per cent reduction in DMF teeth and a 44.5 per cent reduction in DMF surfaces of 139 participants. The age range was 18 to 40 years. The subjects had received five four-minute applications of one per cent sodium fluoride. However, the validity of these results must be questioned, as the half-mouth technique was used in this study.

Day[41] in 1951 reaffirmed the negative results of Arnold, Dean, and Singleton[39] with sodium fluoride. Day's non-significant differences occurred in spite of his application of two per cent solutions rather than one per cent solutions, and his provision for four applications within a maximum of eight days, rather than one application in total.

Rickles and Becks[42] in 1951 showed a two-year reduction of DMF teeth (18.8 per cent) and DMF surfaces (36.8 per cent) in 47 young adults aged 22 to 34 years. They had made four-minute applications of two per cent sodium fluoride solutions.

Kutler and Ireland[43,44] published in 1952 and 1953 the results of sodium fluoride topical applications on a group of 147 medical students having a mean age of 24.6 years. Using four applications of two per cent sodium fluoride within a two week period, they measured a 13 per cent increase in DMF surfaces at the end of one year. However, their research was predicated on the half-mouth technique.

Finally, Carter and others[45] in 1955 compared the results of four 4-minute applications of two per cent sodium fluoride in 60 women in the armed forces between 19 and 39 years of age, with the results of placebo applications in 88 other women in the military service of the same age range. They noted a DMF teeth reduction after one year of only 9.1 per cent in the group receiving the fluoride, compared with the control group.

Three studies concerning the effect of sodium fluoride on primary teeth will next be noted. Ast[46] in 1950 used the half-mouth technique to arrive at a one-year 22 per cent def surface reduction among 260 subjects

ranging in age from two to seven years. A technique of multiple applications was used. In the same year, Wittich[47] reported a like reduction of 22 per cent def surfaces. His results, though, spanned two years. Sundvall-Hagland[48] in 1955 published results indicating a 19 per cent def surface reduction after one year.

Two pairs of authors published negative results following topical application of sodium fluoride in areas of adequate communal fluoride levels. Downs and Pelton[49,50] reported in 1950 and 1951 a 0.4 per cent reduction in DMF teeth after one year for 600 subjects aged 6 to 18 years. It should be noted, though, that they used the half-mouth technique of experimental design.

Galagan and Vermillion[51] in 1955 conducted a similar study on 282 children between 7 and 16 years of age. Their one year results revealed a DMF teeth reduction of 8.9 per cent. They also relied on the half-mouth design.

The use of surfaces versus teeth as a clinical measure warrents a brief note. Since each tooth is arbitrarily defined and reported as containing either four or five surfaces, it would seem that analysis of DMF or def surfaces would be more sensitive to subtle clinical trends than would be DMF or def teeth. In other words, a tooth is either decayed or it is not, whereas it may have zero, one, or possibly up to five decayed surfaces.

Abdul-Ghaffar and Muhler[52] in 1959 examined the additive effects of topical applications of sodium fluoride and stannous fluoride for 6- to 15-year-old children in a non-fluoridated area. One group of 185 received

two four-minute applications of eight per cent stannous fluoride; the second group of 182 had only one such topical application, but it was preceded by a four per cent sodium fluoride application. The first group experienced a 78.2 per cent DMF surface reduction; the improvement for the second group was 60.6 per cent.

A series of reports concerning the efficacy of stannous fluoride (versus placebos and versus other topical fluoride agents) will now be examined. Four studies concerning topical application on permanent teeth in areas of optimal fluoride supply will first be considered. Muhler[53] and Abdul-Ghaffar[54] reported in 1958 and 1959 on a study of 600 school children whose lives had been spent in an area of adequate communal fluoride levels. Half received eight per cent stannous fluoride for four minutes every six months for two years, and were compared with the other half who received a placebo application of distilled water at the same intervals. The experimental group experienced DMF teeth reductions (when compared with the controls) of 32.9, 35.1, 33, and 49 per cent after periods of 6, 12, 18 and 24 months, respectively. Corresponding reductions in DMF surface values of 33.7, 26.0, 35, and 37 per cent also were stressed by the authors.

Muhler[55,56] also reported in 1960 on a series of 6- to 17-year-olds receiving semi-annual stannous fluoride applications with the whole-mouth technique. The application procedure was the same as in the study summarized above. He began with 232 subjects and, after 30 months, was left with 78. Their DMF teeth reductions at six months increments (compared with placebos) were 35.2, 34.9, 35.6, 46.4, and 54.3 per cent. Corresponding DMF surface reductions were 35.9, 31.1, 36.1, 35.3, and 49.2 per cent.

Ship, Cohen, and Lester[57], on the other hand, reported a zero per cent change in DMF teeth levels among 897 11- to 14-year-olds one year after a single four-minute application of eight per cent stannous fluoride.

Horowitz and Heifetz[58] published in 1969 a comparison of one-minute application of eight per cent stannous fluoride annually with 30-second applications of 10 per cent solutions of this agent with the same frequency. Their data were collected from 7- to 11-year-olds. In the first group, DMF teeth reductions of 16.9, 20.4, and 20.9 per cent were observed for one-, two- and three-year periods; DMF surface reductions were 10.0, 12.7, and 21.1 per cent. Corresponding figures for the second group were 0.0, 2.2, and 13.5 per cent for DMF teeth; and 0.0, 5.1, and 3.8 per cent for DMF surfaces. The number of participants in each group ranged from 249 to 345.

In the light of the less marked results of Horowitz and Heifetz[59], it is interesting to note their comments concerning other researchers' results in measuring the clinical effects of topical stannous fluoride treatments. In referring to levels of protection ranging as high as 78 per cent as measured by Abdul-Ghaffar and Muhler[52], these authors acknowledged that no other researchers had been able to even approach this degree of DMF surface reduction.

Clinical studies on the topical application of stannous fluoride in areas of fluoride-deficient water supplies will be discussed next.

Slack[60,61] reported in 1955 and 1956 on a three-application per year schedule of two per cent stannous fluoride. Each application lasted three to four minutes. In the first study, 328 children revealed a 24.9 per cent decrease in DMF surfaces after one year. No age range was given by the authors. In the second investigation, 299 six-year-old children demonstrated a DMF teeth reduction of 31.7 per cent after one year and 24.9 per cent after two. Corresponding DMF surface reductions of 33.2 and 29.2 per cent were noted. In both of these studies, each participant served as his own control. Another methodological problem concerned the investigator's choice of teeth; he consistently used the left side for controls and the right side for topical applications.

Howell and others[62] in 1955 compared applications of two per cent solutions of sodium fluoride and stannous fluoride. Sodium fluoride was applied four times within three weeks, with the applications repeated every three years, while single applications of stannous fluoride were made semi-annually. Comparing each agent with placebo topicals, they noted a 36.3 per cent DMF surface reduction with sodium fluoride, and a 58.8 per cent DMF surface reduction with stannous fluoride. The 58.8 per cent value was attained while applying the solution once and permitting it to dry out on the teeth for four minutes. When they applied the stannous fluoride agent continually for four minutes, the authors observed a 65.5 per cent reduction. The results were derived from two-year observations. Reductions in DMF teeth for the two methods of stannous fluoride applications

were 83.1 per cent (applied and permitted to dry over four minutes) and 60.9 per cent (applied continually for four minutes). Twelve hundred children and adolescents participated in this study.

McLaren and Brown[63] also compared two per cent solutions of stannous fluoride and sodium fluoride for clinical efficacy in 1955. Using about 400 children aged 6 to 11 years old, they measured one- and two-year DMF surface reductions of 46.3, and 18.9 per cent with the stannous agent, and 38.6 and 17.6 per cent with the sodium agent.

Muhler[64], using one application of eight per cent solutions, reported three-month reductions in 45 subjects of 45.0 per cent (DMF teeth) and 48.0 per cent (DMF surfaces). Participants in this 1957 study were six to 15 years old.

Nevitt, Witter and Bowman[65] reported in 1958 on a study similar to those of Howell and others[62] and McLaren and Brown[63]. These authors found 15-month reductions in DMF teeth of 44.4 per cent with stannous fluoride and 35.9 per cent with sodium fluoride. Corresponding DMF surface reductions were 29.0 and 22.6 per cent. Six hundred nine to 14-year-olds participated in this program. Although it would seem from these results that the two agents were about equally successful, the authors' acknowledgement that they used the half-mouth control technique raises some doubt about the veracity of these figures.

Jordan, Snyder, and Wilson[66,67] used annual whole-mouth applications on 510 adolescents aged 12 to 15 years old. One- and two-year DMF teeth reductions of 20 and 14.1 per cent were reported. Reductions in DMF surfaces were 37.9 and 38.3 per cent.

In Muhler's[55,56] previously mentioned report on clinical successes with stannous fluoride in fluoridated areas, he noted concurrent successes in a non-fluoride area. These six to 13-year-old subjects also received four-minute applications of stannous fluoride semi-annually. Six-month reductions in DMF teeth and DMF surfaces of 51.2 and 52.2 per cent were claimed. One-year corresponding reductions for these 250 children were reported at 54.8 and 51.9 per cent.

In 1960 Rothhaar[68] measured three-year and five-year DMF surface reductions of from 60 to 100 per cent in 350 children treated with eight per cent solutions for four minutes every six months. He felt that the extent of the range was dictated by the varying caries activity rates of the children in the study prior to the beginning of his evaluation period. Overall, the values he gave are the highest reported in this review of literature.

Law, Jeffreys, and Sheary[69] reported in 1961 on a comparison of two per cent sodium fluoride (applied over four session two to seven days apart), two per cent stannous fluoride (given on the same schedule), and eight per cent stannous fluoride (given but once). About 823 children aged 7 through 13 years participated in this study. One-year reductions in DMF teeth of 35.8, 33.8, and 18.8 per cent were reported for the three experimental groups in the above order, while corresponding DMF surface reductions over that time were 35.8, 31.6, and 24.9 per cent. The subjects were their own controls, as the authors used the half-mouth technique.

In the same year, Mercer and Muhler[70] reported decreases of 50 and 51 per cent, respectively, in DMF teeth and DMF surfaces one year after one application of eight per cent stannous fluoride. Two applications of this agent led to one-year reductions of 53 per cent in both DMF teeth and DMF surfaces. Six hundred 6- to 14-year-olds contributed to the data in this study.

Peterson and Williamson[71] in 1962 reported on a two-year study among 111 children aged nine to 13 years old. Their participants received four-minute applications of eight per cent solutions once a year; they reported a 26.2 per cent reduction in DMF teeth and a 24.2 per cent reduction in DMF surfaces at the completion of this study.

In the same year, Salter, McCombie, and Hole[72] reported on a comparative study of the efficacy of one and two applications of eight per cent stannous fluoride solutions. The two-application schedule was on successive days. Six- and seven-year-old children were examined. One-third of the 360 subjects served as controls. After one year, the children receiving one fluoride application had a reduction in DMF teeth of 64.7 per cent compared with controls, while the children receiving two applications had a 29.4 per cent DMF teeth reduction. Corresponding DMF surface reductions of 55.8 and 29.5 per cent for the two groups were reported. The authors indicated that the 64.7 and 55.8 per cent values were significant, while the 29.4 and 29.5 per cent figures (for the children receiving fluoride on two successive days) did not represent significant improvements over controls. No explanation was given as to why the second treatment should apparently reduce the overall fluoride efficacy.

Gish, Muhler, and Howell[73-77] compared sodium fluoride and stannous fluoride in a five-year clinical study ending in 1962. Two per cent sodium fluoride was applied by the Knutson[78-80] technique, viz.: four times, two to seven days apart, repeated every three years. Eight per cent stannous fluoride was applied annually, as outlined by Muhler in 1958[81]. By both DMF teeth and surface indices, the stannous fluoride agent was consistently coupled with better results than the sodium fluoride topical. Evaluations were made eight, 24, 36, 48 and 60 months after the initiation of the study. Subjects in the stannous fluoride treatment group had DMF teeth scores 34, 29, 30, 26, and 30 per cent lower than subjects in the sodium fluoride group; corresponding DMF surface differences were 38, 32, 31, 28, and 35 per cent.

Harris[82] reported 23.6 per cent three year reductions in DMF teeth values for 212 young people receiving one application of an eight per cent solution of the agent. The children in this 1963 study served as their own controls, however.

A comparison of 30-second topical applications of 10 per cent stannous fluoride with eight per cent four-minute applications was published in 1964 by Mercer and Muhler[83]. All participants received applications at six-month intervals. Their results would seem to indicate no significant difference between the two techniques. One observer measured reductions in DMF teeth from 84.3 to 66.7 per cent for the 30-second/10 per cent group from six months to two years, and corresponding reductions of 84.3 to 67.8 per cent in the other group. Reductions in

DMF surfaces as measured by this observer were comparably similar across the two experimental categories. The other observer, working with groups about 50 per cent larger than the first, reported lower overall reductions in DMF teeth and surfaces (on the order of 40 to 50 per cent), but he recorded a similar consistency between the efficacies of the two treatments.

Muhler, Stookey, and Bixler[84], working with about 100 six- to 13-year-olds, found reductions in DMF teeth of 55.0, 52.0, and 48.9 per cent after three, six, and nine months. Corresponding DMF surface values were 44.0, 53.0, and 54.8 per cent. These authors also applied an eight per cent solution of stannous fluoride for four minutes every six months for this 1965 study.

The 1965 results of Wellock, Maitland and Brudevold[85] contast sharply with those of many other investigators. One year after a single four-minute application of eight per cent stannous fluoride, they found an increase in DMF teeth of 9.0 per cent. They reported a zero per cent change in DMF surfaces. This study was composed of 211 eight- to 12-year-olds.

In the same year, Torell and Ericsson[86] reported on a two-year study of about 300 10-year-olds in which four applications of two per cent sodium fluoride led to a 19.8 per cent reduction in DMF surfaces; eight per cent stannous fluoride - applied only once - was coupled with only a 3.5 per cent reduction by this index.

Caprioglio and Resta[87] reported in 1967 on two- and three-year results of eight per cent stannous fluoride applications to 489 participants. In this study, there were DMF teeth reductions of 47.5 and 39.5 per cent

after two and three years. DMF surface reductions for these time periods were measured as 38.5 and 40.2 per cent. The authors provided annual applications of an eight per cent solution.

Horowitz and Lucye[88] published negative results with the use of four-minute topical applications of eight per cent stannous fluoride. One goal of their 1967 study was to compare one- and two-application schedules of this agent. After two years, they noted a 27.4 per cent increase in DMF teeth among the 259 eight- to 10-year-olds in the one-application group. The 223 participants in the other group exhibited a 2.7 per cent increase. Corresponding DMF surface increases of 28.2 and 8.3 per cent were reported.

Averill and co-investigators[89,90] published two year results in 1967 of a comparison of three topical agents. Examining about 600 children aged seven to 11 years, they found no significant reductions in DMF teeth or surfaces with either two per cent sodium fluoride, four per cent stannous fluoride, or two per cent phosphate-buffered sodium fluoride.

Cartwright, Lindahl, and Bawden[91], comparing four applications of eight per cent solutions of stannous fluoride with acid phosphate fluoride in 172 children and adolescents, reported a 37.5 per cent reduction in DMF teeth for the stannous fluoride group. These 1968 results were two year values.

Cons and Janerich[92] offered two-year data in 1969 for their clinical comparison of three applications of eight per cent stannous fluoride with four applications of two per cent sodium fluoride. Reductions in

DMF teeth of 7.5 and 17.2 per cent were reported for stannous fluoride and sodium fluoride; DMF surface reductions among the 520 participants were 7.2 and 11.2 per cent.

The preceding 24 studies dwelt on topical protection of permanent teeth. The four that follow examined the effect of topical fluorides on the primary dentition.

McDonald and Muhler[93], investigating in an area of optimal communal fluoridation, reported one-year data for 300 subjects in 1957. The half who had received two per cent sodium fluoride four times over two weeks exhibited reductions in def teeth and def surface of 21 and 12 per cent, respectively. The other three to twelve year olds had been treated with four per cent stannous fluoride by the same two week regimen; def teeth and surface reductions for them averaged 57 and 37 per cent. The authors concluded from these data that stannous fluoride was more effective than sodium fluoride in reducing dental caries in primary teeth.

The other three studies on primary teeth were in areas lacking adequate drinking water fluoride levels. Compton and associates[94] provided one application of eight per cent stannous fluoride to 112 preschool children and in 1959 recorded a 28 per cent reduction in def surfaces after one year.

Four of the five authors[95] in the previously mentioned study reported two years later on a similar study of preschool children. In this case, stannous fluoride topicals were repeated semi-annually. After one year, the

150 participants revealed a mean def surface reduction of 14.8 per cent. Two year reductions for 121 subjects averaged 25.1 per cent.

Salter, McCombie, and Hole[72], in their previously discussed 1962 paper, also measured the effect on primary teeth of one versus two applications of stannous fluoride. One application of an eight per cent solution led to one-year reductions in def teeth and surfaces reductions of 45.2 and 42.4 per cent. Participants receiving two applications had reductions of 35.6 and 42.8 per cent, as measured by def teeth and surfaces, respectively.

Three studies have examined the use of stannous fluoride topical agents in adults. The Muhler[64] paper of 1957 reported on the use of semi-annual four-minute applications of 10 per cent stannous fluoride in a group of 37 persons ranging in age from 17 to 34 years. After one year there was a DMF teeth reduction of 39.0 per cent and a DMF surface reduction of 15.0 per cent.

Muhler[96] used the same technique in a college population of 228, ranging from 17 to 38 years old. Here the one-year DMF teeth and surface reductions were 24 and 16 per cent. Muhler's results stand in contrast to those of other authors[39,41,43-45] who have shown discouraging figures when using sodium fluoride topical solutions in adult groups.

Harris and associates[97] indicated less promising results in their 1964 paper. Ten per cent stannous fluoride again was applied to adults, but only one four-minute application was provided. One examiner measured a 15.0 per cent reduction in DMF surfaces after one year, while another

observed only a 7.4 per cent change in that direction. About 300 subjects were examined in this project.

The authors of all clinical studies cited in this review have measured results either in terms of changes in the incidence of dental caries (as with DMF surfaces or DMF teeth) or as a function of an indirect measure of fluoride content in the outermost layers of enamel. The authors in the next four papers have indicated a correlation between these two forms of measurement. All of these studies permit us to use enamel fluoride uptake values as an indication of the potential of fluorides for the prevention of dental caries.

In 1971 Peterson and Mellberg[98] reported on three-year results of an acidulated fluoride paste self-application program among sixth- and seventh-graders in an area without communal fluoride supplies. Two hundred participants had used this prophylaxis paste, while a like number of children brushed with a non-fluoride placebo paste. The experimental group had DMF teeth and DMF surface rates 21 and 14 per cent lower than those of the controls. Exfoliated primary teeth were measured at the five micron depth for fluoride content: the mean value for the experimental group was 840 ppm; the controls averaged 702 ppm.

Also in 1971, Keene, Mellberg, and Nicholson[99] reported on caries experience and fluoride content of enamel in a series of Navy recruits. The 108 subjects were grouped by caries experience: 47 men in Group 1 presented no history of caries; 31 men in Group 2 had a mean number of

DMF teeth of 8.5 (and a range of 5 to 11); the remaining 30 men forming Group 3 averaged 16.4 DMF teeth (with a range of 12 to 26). Enamel biopsies of right and left maxillary central incisors for these three groups revealed mean fluoride values of 3493 and 3424 ppm in Group 1, 2438 and 2160 ppm in Group 2, and 1957 and 1931 ppm in Group 3. Corresponding enamel depth means were 2.5, 3.1, 3.1, 3.8, 3.7, and 3.5 microns.

Mellberg and associates[100] in 1974 reported on fluoride concentration at five microns and DMF surface experiences as functions of the number of consecutive school days in which acidulated fluoride gel was applied to ten- and 11-year-olds in a fluoridated area. The topical agent was applied for either 25, 10, or 5 days to 704 subjects; 685 others served as controls. Fluoride uptakes were measured as 3800, 3000, and 1800 ppm in the three experimental groups; the values stabilized by the end of the study to 2600, 1900, and 1380 ppm. Corresponding DMF surface value reductions (relative to controls) were 29.9, 27.8, and 16.5 per cent.

DePaola and others[101], also in 1974, as a result of their observation of 1447 children aged 12 to 15 years (from both fluoridated and fluoride-deficient areas), stated that "the relation between corrected log F (as measured by enamel biopsy) and $\sqrt{DMFS}$ was found to be simple and statistically powerful". Their formula was: $\sqrt{DMFS} = -1.236$ (corrected log F) + 17.612. The authors did not elaborate on the adjustment made to fluoride concentration values.

Forrester[13] and Lobene et al[102] proposed that any clinical evaluation of caries following stannous fluoride treatment potentially carries with it a serious inadequacy in analysis: both authors felt that stannous fluoride can mask incipient interproximal lesions in radiographs. Since this misleading condition has no counterpart with other types of topical fluoride applications, results on any of the above stannous fluoride studies may be biased to some extent. Neither of the two authors substantiated his claims by citing specific retrospective studies; they take a more anecdotal stance.

Kamen and Schmee[103] and Viohl[104] commented recently on the potential errors attributable to use of only one person as the evaluator of dental caries. Kamen and Schmee suggested that while the assumption of a constant bias on the part of one examiner (encompassing all experimental categories) might be valid, the power of a statistical test would be reduced even if the errors were to cancel out. Therefore they advised using two examiners and in the case of disagreements, supplementing these two opinions with that of a third evaluator. Viohl felt that two evaluators' judgments diverged on about three per cent of the teeth being measured.

Stookey's[105] comments sum up the previous studies by stating that stannous fluoride topical agents provide significant benefits in caries prevention to all segments of the population, while sodium fluoride is useful to a more limited portion of our society (children reared in the absence of communal fluoride) and acidulated systems require a more thorough series of investigations.

III. Prophylaxis Compound Studies

Jordan and his associates[106] claimed in 1946 that the clinical effect of sodium fluoride topical treatments is enhanced when the treatments are preceded by a prophylaxis. This opinion was reiterated by Knutson, Armstrong, and Feldman[79], in 1947. In Muhler's[107] 1959 review, he applied this same thought to the efficacy of stannous fluoride as a topical agent.

It followed that some investigators would measure the effects of incorporating of fluoride agents into the prophylaxis paste itself. Four such papers describing the incorporation of sodium fluoride are touched upon here. Bibby and others[108] published the first paper on this topic in 1946. A flour of pumice mixture of one per cent sodium fluoride was buffered with acetic acid to a pH of 4.0. Applying the paste three times in one year to six- to 14-year-olds, they measured a 42 per cent reduction in DMF surfaces. They noted, however, that this figure was not significant. Applying the same paste twice in one year to 95 other children, they recorded a 25 per cent reduction in DMF surfaces.

Two years later, Bibby[109] recorded no reduction in DMF surfaces after one year for 250 people who had been similarly treated every four months.

Wellock and Bibby[110] reported in 1954 likewise negative results for 200 ten- to 12-year-olds who had been treated every four months and 82 others within the same age range who had been treated every six months.

On the other hand, DePaola[111] in 1967 stated that he had measured a 31 per cent dental caries reduction in children one year after treatment with a prophylaxis paste containing a 3.3 per cent sodium fluoride hydrofluoric acid.

Other researchers examined the effect of adding stannous fluoride to prophylaxis pastes. Papers discussing pastes other than those based on zirconium silicate will be considered first. Segreto and Harris[112] performed an *in vitro* analysis of a mixture of equal amounts of stannous fluoride and pumice. The authors did not state whether the proportion was by weight or volume. Measuring formic acid decalcification, these authors concluded in this 1959 study that the mixture afforded 50 to 60 per cent protection.

Gish, Howell, and Muhler[113] also tested responses to a 50 per cent stannous fluoride prophylaxis paste. They did not identify the other contents of the paste, and said that they discontinued this 1961 study due to undesireable side effects on the population.

Segreto, Harris, and Hester[114,115] in the same year reported on an *in vitro* study of 40 per cent stannous fluoride in a silex-silicone system. They determined that it was 74 per cent more effective than a five-minute topical application of 10 per cent stannous fluoride. The authors attributed this difference to the cleaning action of silex and the removal of saliva by silicone. However, the same authors and others[116,117] reported in 1960 and 1961 on studies of clinical taste responses. The

reactions by 412 subjects indicated that this factor was a barrier to patient acceptance. Systemic and gingival reactions also were observed.

Segreto, Harris, and Hester[118] and Hester and others[119] claimed in 1960 and 1961 to have circumvented the side reactions of previous studies[116, 117] by reducing stannous fluoride content in their compound to 20 per cent.

In 1963 Peterson, Jordan, and Snyder[120] reported on a clinical study with a 17.5 per cent stannous fluoride/silex-silicone paste. They claimed a two-year DMF surfaces reduction of 34.2 per cent and a two-year DMF teeth reduction of 27.9 per cent, both compared to a control paste. All treatments were made annually.

In contrast to these studies, Hennon and Muhler[121], in reviewing their studies of a silex-silicone-fluoride paste, concluded that this mode of protection was unsatisfactory, as it precluded subsequent topical stannous fluoride applications.

Horowitz and Lucye[88], in their previously mentioned 1967 paper, discussed the use of 8.9 per cent stannous fluoride in lava pumice. Their two-year results with 227 eight- to 10-year-olds receiving annual applications indicated a five to six per cent increase in DMF teeth and DMF surfaces.

Muhler, Boyd, and Van Huysen[122] in 1950 claimed that certain zirconium salts increased the ability of stannous fluoride to reduce enamel solubility in weak organic acids. Dudding, Stookey, and Muhler[123] in

1965 stated that zirconium silicate had four properties contributing to its desirability as a base for a prophylaxis paste. It was a good cleaner; it generated relatively little frictional heat; it caused minimal abrasion to enamel and dentin; and it was a good polishing agent (and thus retarded pellicle and plaque formation). Stookey, Hudson, and Muhler[124] reported on a number of abrasives in 1966; in this *in vitro* study, dry calcium carbonate was used as a standard. Zirconium silicate was shown to have superior polishing properties as compared to 14 tested commercial agents and pastes, and to 11 of the 12 other laboratory abrasives. The only better polisher was tin oxide, but this material was a very poor cleaner. The authors noted also that the degree of polishing of any given agent was a function of length of application, particle size (less than ten microns in diameter being best), powder/water ratio (5.0 grams per 0.3 milliliters) and method of preparation.

Shannon[125] demonstrated in 1966 that stannous fluoride-zirconium silicate paste efficacy was proportionate to the concentration of stannous fluoride in the paste. He used concentrations ranging from 0.64 to 4.80 per cent stannous fluoride in his *in vitro* acid decalcification test.

Stookey and associates[126] in 1967 demonstrated no gingival toxicity with the use of zirconium silicate, calling the material "inert".

Kelley[127] commented in 1967 on the *in vitro* compatibility of this abrasive with stannous fluoride, stating that it was no more reactive with

stannic and fluoride ions as provided by stannous fluoride than was flour of pumice, lava pumice, fine pumice, or silex. In 1969, Kelley, Stookey, and Muhler[128] stated that zirconium silicate was more compatible with stannous fluoride than was lava or flour of pumice.

Further observations on this system came from Whitehurst, Stookey, and Muhler[129] in 1968. They compared a commercial preparation of zirconium silicate with 30 other compounds. This preparation was fifth of 31 in *in vitro* cleaning. It was best of the 31 in enamel polishing. It was 16th in rank order of human and bovine enamel abrasion. It ranked 15th and 19th in human and bovine dentin abrasion. It was third of 21 in reducing *in vivo* enamel solubility; and, finally, it was best of seven in the reduction of *in vitro* enamel solubility.

Muhler and Stookey[130] reported the high polishing and low enamel and dentin abrasion characteristics of a paste made of zirconium silicate in combination with tin oxide. The best polishing properties were found when these compounds were mixed in a 92.5:7.5 proportion. They also found that the cleaning properties of this combination were as acceptable as those of zirconium silicate alone. Measuring phosphorus loss as an indicator of *in vivo* enamel solubility, they found a 77.3 to 82.0 per cent decrease when stannous fluoride was incorporated into the paste.

Four clinical studies confirmed the efficacy of zirconium silicate. Muhler, Dudding, and Stookey[131] in 1964 found it better than four of pumice or lava pumice in removing stain. The agent also was more effective than levigated alumina, tin oxide, insoluble metaphosphate, and pumice

in retarding pellicle reformation. In agreement with Kelley's[127] concurrent report, the authors of this paper found zirconium to be highly compatible with stannous fluoride.

A number of self-application studies have proven the efficacy of a stannous fluoride-zirconium silicate prophylaxis paste. Muhler[132] in 1968 reported on the use of this paste annually by 123 six- to 15-year-olds in an optimal fluoride area. The paste with stannous fluoride exceeded the paste without the fluoride agent in reducing caries activity, as measured by DMF teeth and surfaces. The relative reductions were reported as 19.1 and 35.0 per cent, respectively. In an analogous study of 395 children in a community of inadequate water fluoride levels, the corresponding measures of superiority of the paste with stannous fluoride were 32.9 and 33.9 per cent. Under all experimental conditions, the children were given five grams of the appropriate paste and permitted to use it for five minutes.

In another report in 1970, Muhler and co-workers[133] noted after one year a 41 per cent DMF teeth superiority and a 64 per cent DMF surface advantage of the stannous fluoride-supplemented prophylaxis paste. The area of study had inadequate communal fluoride levels (less than 1.0 ppm).

In a more recent paper, Gish and his associates[134] provided three-year results of self-application procedures in areas of both adequate and deficient communal fluoride levels. In the optimal fluoride area, dental caries reductions of 30.1 and 24.7 per cent (DMF teeth and surfaces) compared with controls were reported. Reductions in the low-fluoride

area in this 1975 study were 31.6 and 37.4 per cent for DMF teeth and surfaces, respectively.

The experimental paste used in the last three studies, commercially available as Zircate Treatment Paste*, had the following ingredients, according to Muhler[132]: $ZrSiO_4$ 48.63 per cent; SnF_2 9.00 per cent; NaH_2PO_4 9.00 per cent; SnO 5.40 per cent; H_2O 12.16 per cent; humectants 11.29 per cent; binders 1.78 per cent; and flavor and sweetening agents 2.74 per cent.

Stookey[105] and Gish[134] stated that the highest degree of fluoride protection against caries can be obtained only through a three-sided approach: through use of a prophylaxis paste containing stannous fluoride; through topical application of stannous fluoride, and home use of a stannous fluoride dentifrice. The importance of this three-sided approach has been borne out by the work of Mericle and Muhler[135] (although the stannous fluoride prophylaxis paste in their laboratory animal study had a pumice abrasive), Bixler and Muhler,[136,137] Gish and Muhler[138], and Scola and Ostrom[139,140].

This review of the literature indicates that periodic topical applications of stannous fluoride reduce dental caries rates in all types of populations, and that in *in vitro* studies they appear to improve the crystallinity of enamel. It also indicates that cleaning of teeth prior to topical applications of stannous fluoride enhances the effects of the

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* L.D. Caulk, Inc., Milford, Delaware.

fluoride agent, that zirconium silicate is an excellent cleaning and polishing agent, and finally that incorporating stannous fluoride into a prophylaxis compound (particularly zirconium silicate), offers protection from caries. Therefore, if a zirconium silicate prophylaxis is to be followed by a four-minute application of 10 per cent stannous fluoride, one may ask whether the presence of nine per cent stannous fluoride in the paste provides an added effect to the topically-applied stannous fluoride. This question could be answered by measuring clinical caries experience or some aspect of enamel structural change. Guided by a modification of the in *in vivo* enamel biopsy technique of Hotz and others[12] an attempt was made in this study to answer the question by means of measuring enamel uptake of fluoride as a function of several different prophylaxis and topical fluoride application regimens.

METHODS AND MATERIALS

Before the clinical data were collected, the modified Hotz biopsy technique was standardized in an *in vitro* setting. Three problems needed to be solved. First, fluoride had to be removed from the biopsy materials, since failure to do this would give misleading readings. Second, the volume of tooth structure necessary to provide an adequate amount of fluoride for replicable measurement had to be determined. Finally, it had to be established that a well circumscribed area of tooth structure could be decalcified to a uniform depth, thus giving a consistent volume. Glass fiber filter paper* was used for the biopsies. It was chosen over more conventional filter paper due to its handling properties: since it was soft, pliable, and non-brittle, it could be readily adapted to a slightly concave or convex surface without leaving space gaps. However, in their manufacture, the 3.7 cm diameter discs had been bleached with hydrofluoric acid. Discs measuring 3.175 mm in diameter elicited readings of about 500 to 600 ppm fluoride. This fluoride was removed by washing the 3.7 cm diameter discs (the size as packaged) three at a time in a Buchner filter. Three 40 ml aliquots of 4N perchloric acid were introduced, followed by two like volumes of Total Ionic Strength Adjusted Buffer (TISAB), essentially an acetate buffer system, and then three washes of a similar volume of deionized water. Immediately after pouring the second of the three $HClO_4$ washes, the vacuum was released,

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* Grade 934 AH, Reeve Angel Co., Clifton, New Jersey.

permitting the acid to remain in contact with the papers for 15 minutes. The vacuum was then resumed. This series of eight washes was repeated for four additional cycles. Each group of three papers was dried at 60°C for 30 minutes; they were then tweezed apart. Using an office paper punch, the 3.7 cm diameter discs were converted into 3.175 mm diameter discs and stored in a clean glass container.

The TISAB solution used for these washes and subsequent dilution of samples was prepared as follows: a 1500 ml beaker was used to mix 600 ml deionized water, 116 gm NaCl, and 114 ml acetic acid. Eight grams *trans*-1,2-diaminocyclohexane-N,N,N',N'-tetracetic acid monohydrate 98% (CDTA, 1,2-cyclohexylenedinitrilotetraacetic acid) was dissolved in about 100 ml 1.5N NaOH. The CDTA/NaOH solution was added to the content of the 1500 ml beaker. After verifying the pH of this mixture to be 3.4, about 250 ml 6N NaOH, or a quantity sufficient to elevate the pH to 5.35,was added. The beaker contents were placed in a two liter flask; beaker rinsings and deionized water sufficient to bring the volume to 2 liters were added. After cooling, the volume was corrected to 2 liters.

A 3.175 mm diameter disc size was chosen on the basis of dental anatomy. It was felt that a disc of this size could be readily confined to the mesial or distal facet of the facial surface of a cuspid. Similarly, it could be applied to the mesial aspect of the facial or lingual sufface of a partially-erupted permanent molar without touching the gingiva, going into the buccal groove, or being layered over the cusp

onto the occlusal surface. Otherwise, the area of decalcification would not accurately reflect the true area of the disc.

Bovine teeth were used in the preliminary *in vitro* studies. The roots were removed and the crowns were cleaned of soft tissue and debris. Then they were pumiced on a rag wheel, etched for 30 seconds with 2N $HClO_4$, and repumiced. Lastly, they were imbedded in autopolymerizing acrylic cubes so that only the facial surface of each crown was exposed.

Various volumes of different concentrations of $HClO_4$ were evaluated in the discs which were applied for various lengths of time to pumiced and polished bovine tooth surfaces. To stimulate clinical conditions better, some of the bovine teeth were immersed in 10 per cent aqueous stannous fluoride at 37°C for four minutes. All bovine teeth were then immersed in a solution commonly known as "artificial saliva" for 72 hours, changing the solution every 24 hours.

"Artificial saliva" is a solution of mineral composition resembling that of saliva, but lacking its organic components. It is used to keep the teeth in an environment close to their natural one, and to remove fluoride loosely bound to the enamel surface. To prepare the solution nine grams of $CaSO_4 \cdot H_2O$ was diluted to 4.5 liters with deionized water. Four salts were separately diluted to 1.0 liter aqueous solutions: 11.25 g NaCl, 4.545 g KCl, 9.315 g KH_2PO_4, and 11.007 g Na_2HPO_4. To a 5-gallon carboy containing 17 liters deionized water the following was added: 450

ml of the $CaSO_4 \cdot H_2O$ solution, and 100 ml each of the other four stock solutions (in the order listed above). The container was then filled with deionized water to complete 18 liters. The pH was verified to be between 6.9 and 7.2.

It was determined that about 2.5 µl of the liquid saturated the discs. A 10 µl micropipette* calibrated in a 0.2 µl gradations was used to apply this volume to the discs. In testing other volumes of liquid, it was found that with volumes much greater than 2.5 µl an excess was expressed onto the tooth during application of the disc, thus preventing control of the surface area of the biopsy site. Noticeably lesser volumes of acid wetted the discs inadequately and thus would also give misleading values. Biopsies were performed on bovine teeth using different acid concentrations and application times.

In all cases the discs were applied with a clean cotton forceps. As predicted[141], there was a direct relation between length of time of acid contact and thickness of decalcification layer.

A 5-second application of 2N $HClO_4$ to the stannous fluoride-treated surfaces and a 5-second application of 1N $HClO_4$ to the surfaces not treated with a fluoride solution gave fluoride values which were measurable and provided reasonably uniform decalcification thicknesses. The need for a greater concentration of acid to decalcify a fluoride-treated surface can be explained by a predicted interference of the stannous ion

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* Model 701, Hamilton Co., Whittier, California.

with the decalcification process. Perchloric acid was used since weaker acids require a much longer time to decalcify enamel to an adequate depth[136] and tend to selectively decalcify different ions to different depths[142,143].

A total of 119 participants were chosen for the collection of clinical data. Most of these participants were patients in the pedodontic clinics at Indiana University. About 20 were siblings or friends of these patients. One criterion for selection was the presence of all four primary cuspids and all four permanent first molars. About one-half or more of the anatomic crown of the molars had to be visible. Subjects were excluded from the study if any of these eight teeth were restored with crowns or any material covering more than half of the facial or lingual surface. This case selection permitted at least a minimal surface area for enamel biopsy. Subjects were also excluded if any of the proposed biopsy sites were carious or grossly hypocalcified. It was expected that carious enamel would provide relatively higher baseline fluoride values[144,145] and that carious or otherwise altered enamel would take up fluoride to an extent greater than that of sound enamel[12,146,147]. However, an earlier study[148] suggested that fluoride concentrations in sound and carious enamel were about equal. During the study, a few participants had small, well-circumscribed circular areas of hypocalcification on the facial aspect of their mandibular primary cuspids. These areas were rarely so extensive as to disqualify the child from the study.

The choice of teeth was made to permit comparisons of primary versus permanent and anterior versus posterior dental units.

As predicted by the criteria for their selection, most participants were seven, eight, or nine years old. Of the 89 participants completing the study, the mean age was 8.20 years, with a range of 5.93 to 10.87 years. Forty-eight boys and 41 girls completed the program. Two of the 89 were included in the data analysis, although they missed the second appointment of the sequence of three.

Prior to the biopsies, parents were asked the source of their water supply. From their responses, it was determined that about one-third of the children in the study had grown up drinking water with an adequate level of fluoride. An approximately equal number had an inadequate (less than 1.0 ppm) communal fluoride experience. Of the remaining one-third, some had received a mixed experience with proper communal fluoride supplies, while other could not furnish adequate information. A sample of the information form is provided in Appendix 1. Since the subjects were to be randomly assigned to the treatment groups, and the communal fluoride/non-communal fluoride ratio was about equal, it was decided that this factor should not influence distribution of subjects within the treatment groupings. It should be noted though that the wide range of fluoride experiences within each group contributed to an increased problem of variability when the data were processed.

A brief schema of the five treatments is as follows:

Group I Zircate Treatment Paste* - topical SnF_2 - no rinsing for 30 minutes.

Group II Zircate Treatment Paste - no rinsing for 30 minutes.

Group III Zircate Prophy Paste** - topical SnF_2 - no rinsing for 30 minutes.

Group IV Zircate Prophy Paste - no rinsing for 30 minutes.

Group V Zircate Treatment Paste - topical SnF_2 - rinsing *ad libitum*.

Three appointments were needed for each child. On the first appointment, four baseline biopsies were taken. Eight teeth were available, and each tooth offered both a lingual and a facial biopsy site. The four sites per appointment had been randomized prior to the clinical appointments, as per Appendix 2. The randomization dictated that at each appointment, two primary cuspid and two permanent molar surfaces would be biopsied. By this process, twelve of each subject's 16 available surfaces were used in the course of the experiment. The preselected surfaces were cleaned of debris and dried with

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* L.D. Caulk Co., Milford, Delaware, Lot number 741341

** L.D. Caulk Co., Milford, Delaware, Lot number 72347

gauze sponges. A washed and dried filter paper disc was saturated with 2.5 µl of 1N $HClO_4$. With the clean cotton forceps it was applied flush to the tooth surface for five seconds. Standardization of the specific area of application was attempted, namely, midway from the cemento-enamel junction to the incisal edge or cusp tip. It had been predicted that there would be an incisal/cervical gradient of fluoride distribution[149] and it was hoped that this gross attempt at standardization would reduce or eliminate that variable as an interacting factor. The disc was removed and inserted into a plastic vial containing 3 ml TISAB. A dry disc was then wiped over the biopsy site in order to absorb any small quantity of the decalcification solution which might have remained after removing the first disc. This second disc was then added to the same vial as the corresponding first disc. The vial was randomly assigned a coded number to eliminate the need of identifying the subject, date, biopsy site, or treatment group. The procedure was repeated for the other three surfaces.

Concerning the prophylaxis, the paste was applied with moderate pressure using a rubber cup* in a conventional prophylaxis handpiece. All teeth in the mouth were cleaned with this paste, one-half of the mouth at a time. The excess paste was removed by irrigation and high-speed aspiration. The aqueous solution of 10 per cent stannous fluoride was applied to one side of the mouth at a time in the appropriate subjects,

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* Model #277 Screwshank
Teledyne Dental, Densco Division, Denver, Colorado.

keeping all tooth surfaces moist for four minutes. Cotton rolls secured by Garmer Clamps* minimized salivary dilution of the solution. All subjects were permitted to spit out excess solution following removal of the cotton rolls. Subjects in Group V were given a paper cup with about 120 ml water and were told to rinse as desired. A few refilled the cup and rinsed further. All other subjects (Groups I to IV) were told - first in the operatory and then again in the presence of their parent or guardian - to neither eat, drink, nor rinse for at least 30 minutes. Some of these participants were particularly upset with the taste of the solution or paste; a moistened gauze sponge was wiped over the dorsum of their tongue to alleviate this problem without changing their treatment status. All subjects were given a soft bristle toothbrush and a tube of toothpaste, both to be used in place of their regular home care aids from that day until the day of the third appointment. The unmarked tube of toothpaste had been formulated without fluoride, so as to help the control and standardization of fluoride acquisition from other sources subsequent to the first week's treatment.

Seven and 14 days after the first appointment, biopsies were obtained from four different surfaces of each subject. These biopsies provided an indication of fluoride uptake as a result of the particular treatment completed immediately after the baseline biopsies. Different surfaces were used each week since a re-biopsy of any given site would remove components

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* Garmers Dental Instruments, Inc.
Minneapolis, Minnesota.

at a greater depth than for a previously unbiopsied site[140]. The technique at 14 days was identical to that used at seven days, with two exceptions. First, the sites were cleaned prior to application of the discs with an insoluble metaphosphate glycerin paste*. This paste was chosen for its very low abrasion, so as to avoid removal of the fluoride-rich outermost layer of enamel. The same type of rubber cup as before was used for these cleanings. Second, biopsies of subjects in Groups I, II, III, and V were performed using 2N $HClO_4$. One Normal $HClO_4$ was used for Group IV. As noted in the pilot part of this study on bovine teeth, the stannous ion tended to interfere with acid decalcification, and it was felt necessary to try to standardize biopsy depths due to the steep gradient of fluoride concentration in the most peripheral layers of dental enamel[150-153]. It was hoped that this variation in acid concentration would correct for the inhibitory effect of the stannous ion on the action of the acid.

Following the last biopsies, each participant received a prophylaxis and a second topical application of ten per cent aqueous solution of stannous fluoride. It has been cited that decalcified or etched enamel can remineralize[147,154,155] and, in fact, that it will take up stannous fluoride at a rate up to five times that of unetched enamel[146]. Thus it was thought that the very minor alteration that had been produced on the tooth surface by the biopsy procedures would be corrected[12].

The 1060 coded vials were regrouped so that all biopsies of each subject would be analyzed at about the same time, but the sequence within

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* Powder Lot Number 11-456.

each group of 12 would be random, and the sequence from subject to subject would not favor any treatment group.

Chemical analyses were performed first for fluoride and then for calcium.

Fluoride ion analysis was made using an Orion Ionalyzer* with an expanded scale digital millivoltimeter**. The electrode is constructed of single-crystal sections of rare earth fluorides and sealed in a polyvinyl chloride tube filled with 0.1M NaF and 0.1M KCl; electrical contact is made with an Ag-AgCl wire[156,157]. This electrode is very selective to the fluoride ion, and responsive to this ion over five orders of magnitude[156], as low as 10^{-6}M. One milliliter of deionized water was added to each 3 ml sample/TISAB solution. The presence of fluoride was read in millivolts on the expanded scale of the apparatus. A standard curve was constructed using solutions of known fluoride concentration, and used to convert the reading of the biopsy specimens from millivolts to micrograms of fluoride per milliliter.

Calcium ion analysis was made using a Perkin-Elmer Atomic Absorption Spectrophotometer***. One milliliter of each sample solution was diluted with approximately 2 ml $LaCl_3$, and sufficient deionized water to bring the total volume to 10 ml. The $LaCl_3$ solution had been made from La_2O_3 dissolved in HCl to about a 10 per cent solution. The purpose of the

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* Model 94-09 Orion Research Corp., Cambridge, Massachusetts.
** Model 701, Orion Research Corp., Cambridge, Massachusetts.
*** Model 303, Perkin-Elmer, Instrument Division, Norwalk, Connecticut.

$LaCl_3$ was to mask $PO_4^{\equiv}$ in the sample which might otherwise be interpreted as Ca++. The samples were analyzed in approximately the same order for calcium ion concentration as for fluoride. Calcium ion results were reported in micrograms per milliliter. One sample was inadvertently spilled during analysis, reducing the number of specimens to 1059 biopsy vials. Also, 35 blank vials (each containing 3 ml TISAB and two washed but unused filter paper discs) were analyzed for fluoride and calcium. The mean values from the blank vials (0.027 µg/ml for fluoride and 0.46 µg/ml for calcium) were used to correct raw scores.

Raw scores were converted to decalcification thickness (in microns) and fluoride ion concentration (in parts per million). The following formulas describe the conversions:

$$(1)\quad \text{Depth } (\mu) = \frac{(Ca^{++} \text{ raw score } \frac{\mu g}{ml} - 0.46 \frac{\mu g}{ml})\ (6 \text{ ml})\ (1000 \frac{\mu}{ml})}{(7.9173 \text{ mm}^2)\ (2870 \frac{\mu g}{mm^3})\ (.367)}$$

$$(2)\quad F^- \text{ content (ppm)} = \frac{(F^- \text{ raw score } \frac{\mu g}{ml} - 0.027 \frac{\mu g}{ml})\ (10^6)}{(7.9173 \text{ mm}^2)\ (\text{Depth } \mu)\ \frac{(1 \text{ mm})}{1000\ \mu}\ (2870 \frac{\mu g}{mm^3})}$$

The formulas are modifications of those presented by Stearns[158]. In both cases, raw scores were modified by the mean values of Ca^{++} and F^- found in the 35 blank samples (0.46 µg/ml and 0.027 µg/ml, respectively). The

sample area was taken as 7.9173 mm^2. Katz[159,160] has noted that other researchers reported a wide range of Ca^{++} values (as a per cent of the inorganic portion of enamel)[159], varying from 33.60 to 39.4 per cent. His value, by weight, was 36.7 per cent[160]. The value of 2870 $\mu g/mm^3$ represents the density of enamel.

The data are presented in Tables according to groups and fluoride enamel content before treatment and one and two weeks after the treatment. Statistical analyses of the differences were performed by means of the t-test. Since some of the values for both the thickness of enamel removed by the perchloric acid and the fluoride enamel content appeared to deviate inordinately from the mean, it was decided to eliminate those specimens whose values were beyond $\pm$ 2 standard deviations from the mean. This eliminated 86 samples. Tables I and II reflect this change.

RESULTS

Of the 355 pretreatment samples evaluated for fluoride and calcium content, those with values beyond $\pm$ 2 standard deviations from the mean decalcification thickness or mean enamel fluoride value (totaling 31) were removed from consideration. The mean thickness of the remaining 324 pretreatment samples was 2.19 μ (compared with 2.23 μ in the 355 - sample group); the mean fluoride content was 1588 ppm (compared with 1578 ppm).

Pretreatment samples were grouped by biopsy sites (facial versus lingual, permanent versus primary, maxillary versus mandibular, and right versus left) and compared for differences in mean decalcification thicknesses and in mean fluoride content. Significant differences ($p < 0.05$) were found for both criteria in the comparisons between permanent and primary teeth. No other statistically significant differences were found.

As footnoted in Table III, there were no statistically significant differences between the fluoride content of different groups of permanent teeth and between different groups of primary teeth. Thus, permanent and primary teeth were considered separately.

Table III shows mean one-week and two-week uptake values for permanent and primary teeth. One-week uptake values were negative for both permanent and primary teeth in all five treatment groups, ranging from -19 ppm to -421 ppm. Group I values for primary teeth were significantly lower ($p < 0.05$). Two-week uptake values were negative for primary teeth in all five groups (ranging from -3 ppm to -505 ppm), and significantly so ($p < 0.05$) in Groups I and V. For permanent teeth, two-week uptake values

were negative for Groups I and III. None of the permanent teeth uptake values, whether negative or positive, were statistically significant.

Table IV illustrates mean decalcification thicknesses for each type of permanent tooth and primary tooth biopsies. Compared to the thickness dissolved prior to treatment, the mean thickness values increased one week after treatment in Group I, II, and III (permanent and primary teeth) and in Group V (primary teeth only). The range of values was from .22 μ to .76 μ. The increases were significant ($p < 0.05$) for Groups I and III (permanent and primary teeth) and for Group II (primary teeth only). On the contrary, there was a decrease in the thickness values of enamel dissolved in Groups IV and V of permanent teeth (.32 μ and .06 μ) and Group IV of primary teeth (.22 μ). However, these differences were not statistically significant. Mean thickness values increased two weeks after treatment in Groups I, II, and III (permanent and primary teeth) and in Group V (primary teeth only). Here the range of values was from .19 μ to .81 μ. The increases were significant ($p < 0.05$) for Group III (permanent and primary teeth) and for Groups I and II (primary teeth, only). The value of the two-week mean thickness decrease for Group IV permanent teeth (.44 μ) was statistically significant at the .05 level; the decreased values for Group IV primary teeth (.29 μ) and Group V permanent teeth (.24 μ) were not statistically significant.

TABLES

TABLE I

Distribution of 1059 Samples by Treatment Group and Biopsy Week

Group	Pretreatment	One Wk. Post.	Two Wks. Post	Total by Group
I	80	80	80	240
II	72	68	72	212
III	80	80	80	240
IV	64	64	64	192
V	59	56	60	175
Total By Treatment	355	348	356	1059

TABLE II

Distribution of 973 Samples by Treatment Group and Biopsy Week (after removing the samples falling outside ± 2 standard deviations of mean biopsy thickness or mean enamel fluoride content).

Group	Pretreatment	One Wk. Post.	Two Wks.Post.	Total by Group
I	74	73	74	221
II	67	61	67	195
III	71	74	74	219
IV	57	62	58	177
V	55	51	55	161
Total By Treatment	324	321	328	973

TABLE III

Enamel Fluoride Content Before Treatment and One and Two Weeks After Treatment

Group	Type of Tooth	Pretreatment Enamel Fluoride Content** (mean ppm ± S.E.)	1 Week Fluoride Data		2 Week Fluoride Data	
			Mean ppm ± S.E.	Net Fluoride Uptake	Mean ppm ± S.E.	Net Fluoride Uptake
I	Permanent	1839 ± 111	1488 ± 109	-351	1717 ± 115	-122
II	Permanent	1562 ± 110	1391 ± 72	-171	1596 ± 98	34
III	Permanent	1772 ± 123	1353 ± 96	-419	1486 ± 87	-286
IV	Permanent	1680 ± 106	1454 ± 150	-226	2026 ± 150	346
V	Permanent	1604 ± 121	1499 ± 137	-105	1721 ± 107	117
I	Primary	1592 ± 93	1171 ± 70	-421*	1174 ± 95	-418*
II	Primary	1391 ± 118	1098 ± 82	-293	1288 ± 90	-103
III	Primary	1459 ± 116	1136 ± 84	-323	1146 ± 83	-313
IV	Primary	1371 ± 103	1353 ± 143	- 18	1368 ± 132	- 3
V	Primary	1555 ± 116	1240 ± 111	-315	1050 ± 80	-505*

Differences which were statistically significant at p = 0.05 or better (using the t - test) are marked with an asterisk. All other differences were not statistically significant.

** A t-test at p = .05 demonstrated that there were no significant differences between the pretreatment fluoride content of the different groups of permanent teeth. The same was true for the primary teeth. However, several of the mean values for permanent teeth were significantly different from those of primary teeth.

TABLE IV

Thickness of Enamel Removed by the Perchloric Acid Decalcification Before Treatment and One and Two Weeks After Treatment

Group	Type of Tooth	Pretreatment Biopsy Thickness (mean μ ± S.E.)	1 Week Thickness Data		2 Week Thickness Data	
			Mean μ ± S.E.	Difference from mean pretreatment thickness	Mean μ ± S.E.	Difference from mean pretreatment thickness
I	Permanent	1.98 ± .07	2.46 ± .11	.48*	2.17 ± .11	.19
II	Permanent	2.22 ± .10	2.44 ± .12	.22	2.41 ± .10	.19
III	Permanent	2.02 ± .08	2.46 ± .10	.44*	2.52 ± .11	.50*
IV	Permanent	2.02 ± .09	1.70 ± .09	-.32	1.58 ± .10	-.44*
V	Permanent	2.32 ± .10	2.26 ± .10	-.06	2.09 ± .14	-.23
I	Primary	2.26 ± .06	2.86 ± .11	.60*	2.75 ± .09	.49*
II	Primary	2.42 ± .10	2.89 ± .11	.47*	2.86 ± .10	.44*
III	Primary	2.20 ± .09	2.96 ± .08	.76*	3.01 ± .11	.81*
IV	Primary	2.17 ± .09	1.95 ± .08	-.22	1.87 ± .11	-.30
V	Primary	2.35 ± .09	2.69 ± .11	.34	2.76 ± .14	.41

Differences which were statistically significant at p = 0.05 or better (using the t-test) are marked with an asterisk. All other differences were not statistically significant.

DISCUSSION

The purpose of this study was to determine whether a variation in the mode of delivery and quantity of exogenously applied stannous fluoride in children would result in significant differences in the uptake of the agent in enamel. *In vivo* samples of enamel were gathered by a modification of a previously published perchloric acid etching technique; analyses of these samples for calcium and fluoride ions were performed using an ion-specific electrode (for fluoride) and atomic absorption spectrophotometry (for calcium).

These measurements were compared to determine the significance of the differences between mean fluoride uptakes (after one and two weeks) of any pair of treatment groups.

It was apparent that within the wide range of distribution of thickness and fluoride concentration values, there were some which were markedly aberrant. It was felt that unless the population sample was modified in some way, the extreme variances within any type of group would make tests for significance inordinately rigorous. It was decided to eliminate those samples falling outside of a range of $\pm$ 2 standard deviations, or about the highest and lowest 2.5 per cent of the population. All 355 pre-treatment biopsies were examined as one group. The mean decalcification thickness value was 2.23 µ. Seventeen values fell outside of a range of $\pm$ 2 standard deviations (0.97 µ - 3.48 µ), and were eliminated from further consideration. The mean fluoride content of the remaining 338 samples was 1578 ppm. Fourteen samples feel outside of a

range of $\pm$ 2 standard deviations (91 ppm - 3065 ppm) and were likewise removed. For the remaining 324 pre-treatment samples, the mean thickness was 2.19 μ and the mean fluoride content was 1588 ppm. The loss of 31 pre-treatment samples was equivalent to about 8.7 per cent of the original 355 samples. It was deemed safe to deal with the initial 355 samples as a whole, since no treatment had been performed on the teeth prior to these biopsies, and any population variabilities were presumably distributed randomly.

A similar pruning of the 704 post-treatment biopsies was planned. When the sample was divided according to all variables, the population cells consisted of about four or five specimens (since there were 16 biopsy sites, two biopsy times, and five treatment groups), and it was therefore virtually impossible to eliminate any aberrant figures. However, since the pre-treatment samples differed significantly in fluoride content only in the primary versus permanent comparison, post-treatment samples were also pooled in terms of left versus right, mandibular versus maxillary, and facial versus lingual. This resulted in a more statistically acceptable cell size of 28 to 40. Each of the population cells was evaluated in the same manner as the 355 pre-treatment biopsies: first eliminating up to three samples per cell on the basis of aberrant thickness values, and then eliminating zero, one, or two samples from each revised cell on the basis of aberrant fluoride concentration values. In this way, the size of the post-treatment population fell from 704 to 649, and it

became possible to consider a less variable, yet totally representative, set of values. Table II shows the result of eliminating of aberrant biopsy samples.

Even with the tailoring of our population pool, samples were observed which seemed, at first glance, intuitively "wrong" in fluoride content levels. The samples were elicited from subjects who consistently and uniformly (in both pre- and post-treatment biopsies, and from all treatment groups) revealed extreme fluoride content readings. Therefore these readings were interpreted as being reflections of the pre-program characteristics of the randomly chosen participants and not necessarily an effect of individual biopsy or measurement errors. It was decided not to further alter the number of specimens by any further elimination of samples, and these subjects were not eliminated.

Table III shows one-week and two-week mean fluoride uptakes for permanent and primary teeth in each of five groups, based on the refined population samples. Also footnoted in Table III are the mean fluoride content differences among permanent teeth pre-treatment groups and primary teeth pre-treatment groups. None of the differences for the intra-group pre-treatment comparisons was significant. These similarities of values were expected, due to the random distribution of participants in the study.

Mean fluoride uptakes were then examined. It was planned to determine whether or not any one- or two-week uptake of any treatment group (among permanent teeth) differed significantly from that of any other treatment

group (among permanent teeth). A similar comparison in the treatment groups composed of primary teeth was planned. Put in a question: "Did the effect of any treatment group differ significantly from that of any other treatment group?" However, seven of the 10 mean uptakes for permanent teeth were negative (none of the 10 changes being significant at the .05 level) and all of the 10 primary teeth mean uptakes were negative (three being significant at the .05 level).

Table IV provided some insight into the paradox of apparent "loss" of fluoride following various fluoride treatments. This table gives the mean decalcification thickness for each type of permanent tooth and primary tooth biopsy. Of the 10 pre-treatment - one week post-treatment differences, five were significantly thicker at one-week. Of the 10 two-week comparisons, four were significantly thicker and one thinner at two weeks. This meant that fluoride content was being measured at significantly different depths from pre- to post-treatment biopsies. Several authors[142,143, 149-153, 161-164] had elaborated on the fluoride concentration gradients in enamel as a function of depth from the surface. The gradient is so steep at the most peripheral layers of enamel that one author[153] referred to it as a "hockey stick curve". With such a steep gradient, no valid comparison could be made of any two populations unless the mean biopsy depths for the two populations were equilibrated. Unfortunately, none of the cited authors had precisely described this gradient within the quite narrow range of

the interest of this study (the most superficial three or four microns of tooth structure). Aasenden and Moreno[161] proposed a mathematical model for this relationship to make corrections of fluoride concentrations when different thicknesses of enamel are dissolved.

In an attempt to apply their mathematical model to the characteristic curve (and thus permit comparison of fluoride values based on disparate biopsy thicknesses), the assistance of a statistician was solicited. He suggested a review of all statistical methods employed by the author. The statistician applied a stepwise regression analysis to the pre-treatment data. He analyzed these data both with and without the "aberrant" 31 samples. The question posed was, "To what extent do the five recognized variables (the four site variables and the decalcification depth values) account for all of the variation in the pre-treatment population sample?" When all 355 samples were assessed, the coefficient of determination was 0.204. When only the 324 samples of the modified population were assessed, this coefficient was reduced to 0.102. The determination coefficients, when squared and multiplied by 100 per cent, denote the percentage of population variation for which the five variables account. Thus, with the 355 pre-treatment samples, 4.2 per cent of the initial variation is identified by the five variables; with the tailored 324 sample pre-treatment population, only 1.0 per cent is identified. Either way, at least 95.8 per cent of the variation is unaccounted for. As a result of this stepwise regression analysis, it was decided that further attempts to equilibrate

the unlike mean decalcification thicknesses would not lead to meaningful results.

In general terms, there are four classes of errors which may have in varying degrees contributed to the results: subject selection, methods of treatment, methods of biopsy, and measurement determinations.

Subject selection will be considered first. The original 119 participants had an extremely heterogeneous history of communal fluoride exposure. As a result, pre-treatment biopsies revealed a broad spectrum of initial enamel fluoride levels. Thus a profound factor of variability was introduced, even with the random assignment of participants to the five groups. Also in regard to subject selection, the quantity of participants definately should have been increased. This is particularly important due to the need for more samples to better describe the fluoride concentration gradient.

There are many facets in the fluoride treatment and biopsy technique which demand refinement. No attempt was made to standardize the pressure and duration of the application of the prophylaxis cups. Occasional salivary dilution of fluoride (in spite of cotton roll isolation) would affect results. The biopsy area may have been poorly controlled due to inadvertent over- or under-saturation of the discs with the decalcification agent. Adaptation of the discs, in spite of their pliability, may not have been uniform. Furthermore, Van der Merwe and Retief[165], examining acid-etched biopsy sites with scanning electron microscopy, claimed in their

study that both biopsy area and thickness were not uniform. These factors might have introduced further variance. The choice of perchloric acid concentrations, predicated on the *in vitro* pilot study with bovine teeth, definitely required further refinement so as to elicit comparable decalcification thicknesses regardless of the type of stannous fluoride treatment. Comparable biopsy thickness across groups would obviate the need for calculating a fluoride concentration gradient. One of the hygienists intermittently used a stop watch calibrated to tenths of seconds to verify the uniformity of the operator's timing of biopsy duration. As best as could be determined by this technique, the times were uniform to within 0.5 seconds. Nevertheless, some variability also was present here. The teeth were dried with air prior to the biopsies and the tongue, buccal mucosa, or lip was reflected away from the biopsy area by the operator's fingers during the sampling. It is very possible, though, that some saliva at times were not kept off the disc. The discs themselves may have retained more fluoride from their manufacture after the perchloric acid/TISAB/water wash than indicated by the blank discs. Participants who were instructed not to rinse may or may not have followed the instructions.

Possible measurement errors include inaccurate measurement of the buffer solution, inaccurate dilution of samples prior to measurement, incorrect reading or transcription of raw values from the laboratory equipment, and calculation blunders in converting raw scores to refined scores.

All of these potential sources for error have the capacity of increasing variance in the results, and thus reducing the possibility of attaining clear cut differences in enamel fluoride uptake as a function of type of treatment. Furthermore, some of these sources of error would tend to decrease the amount of variance that could be accounted for in the stepwise regression analyses, and thus make any results moot.

The study provides numerous points for conjecture and suggests further refinement of biopsy techniques. Only through adaptation of the foregoing mentioned modifications (and quite probably others) will investigators be able to confirm or deny the initially stated hypotheses.

SUMMARY AND CONCLUSIONS

The hypotheses of the study were:

1. When a zirconium silicate prophylaxis paste is applied prior to topical application of 10 per cent aqueous solution of stannous fluoride, the presence of nine per cent stannous fluoride in the paste does not promote a significantly greater enamel uptake of fluoride than when there is no added stannous fluoride in the zirconium silicate prophylaxis paste.

2. Rinsing the mouth with tap water immediately after application of zirconium silicate prophylaxis paste (containing nine per cent stannous fluoride) and topical application of 10 per cent aqueous solution of stannous fluoride does not significantly affect enamel fluoride uptake.

A modification of an *in vivo*, acid-etched enamel biopsy technique was used to measure fluoride and calcium contents of the outermost layer of enamel in first permanent molars and primary cuspids of 89 children. Four sites were biopsied prior to treatment. Treatments involved prophylaxes with a zirconium silicate paste either containing or not containing nine per cent stannous fluoride, followed by either a four-minute topical application of 10 per cent stannous fluoride in an aqueous solution or no

such topical application. Some participants were instructed not to rinse for one-half hour; others were immediately offered unlimited quantities of tap water. Four other sites were biopsied one week after treatment; four more were biopsied two weeks after treatment.

Pre- versus post-treatment differences were measured via a fluoride-sensitive electrode and atomic absorption spectrophotometry.

Results were inconclusive. Enamel fluoride uptakes for most treatment groups appeared to be negative. A stepwise regression analysis of pre-treatment biopsy values indicated that no conclusions, regardless of the nature of the comparative fluoride uptakes, could logically be drawn. Tentative partial explanations, stated in terms of subject selection, methods of treatment, methods of biopsy, and measurement determinations, were offered.

APPENDIXES

ORAL HEALTH RESEARCH INSTITUTE

410 BEAUTY AVENUE

INDIANAPOLIS, INDIANA 46202

317:264-8822

Child's Name_____ Sex_____
(Last) (First) (Middle)

Address_____ Telephone_____

Age_____ Date of Birth_____ Parent's Name_____

How long has your child lived in the Indianapolis area? _____

If your child has not lived in Indianapolis since birth, please list the town or towns he lived in_____

Which do you receive: City Water_____ Well Water_____ (Check One)

I have read the attached letter.

I wish my child to participate in the dental study program. I understand that participation is completely voluntary and that my child is free to withdraw at any time.

Date_____ _____
Parent's Signature

Date_____ _____
Child's Signature

Each child must have a completed consent card to participate.

Appendix 1: Information/Consent Form.

Appendix 2: Example of the Biopsy Site Randomization Pattern

Subject Number	Pre-Treatment Enamel Biopsy: Day 1															
	Facial Surfaces								Lingual Surfaces							
	3	C	H	14	19	M	R	30	3	C	H	14	19	M	R	30
1	x	x					x	x								
2	x		x			x		x								
3	x							x		x					x	
4	x							x			x			x		
5				x	x					x					x	
6				x	x						x			x		
7		x		x	x		x									
8			x	x	x	x										
9									x		x			x		x
10									x	x					x	x
11			x			x			x							x
12		x					x		x							x
13			x			x						x	x			
14		x					x					x	x			
15											x	x	x	x		
16										x		x	x		x	

Subject Number	Post-Treatment Enamel Biopsy I: Day 8															
	Facial Surfaces								Lingual Surfaces							
	3	C	H	14	19	M	R	30	3	C	H	14	19	M	R	30
1			x			x						x	x			
2		x					x					x	x			
3											x	x	x	x		
4										x		x	x		x	
5	x	x					x	x								
6	x		x			x		x								
7	x							x		x					x	
8	x							x			x			x		
9				x	x					x					x	
10				x	x						x			x		
11		x		x	x		x									
12			x	x	x	x										
13									x		x			x		x
14									x	x					x	x
15			x			x			x							x
16		x					x		x							x

Appendix 2 Cont'd.

Subject Number	Post-Treatment Enamel Biopsy II: Day 15															
	Facial Surfaces								Lingual Surfaces							
	3	C	H	14	19	M	R	30	3	C	H	14	19	M	R	30
1									x		x			x		x
2									x	x					x	x
3			x			x			x							x
4		x					x		x							x
5			x			x						x	x			
6		x					x					x	x			
7											x	x	x	x		
8	x	x					x	x		x		x	x		x	
9	x	x					x	x								
10	x		x			x		x								
11	x							x		x					x	
12	x							x			x			x		
13				x	x					x					x	
14				x	x						x			x		
15		x		x	x		x									
16			x	x	x	x										

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ABSTRACT

Fluoride Uptake by Enamel from Stannous Fluoride and Prophylaxis Pastes

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The purposes of this study were twofold: (1) to determine whether applying zirconium silicate prophylaxis paste (containing nine per cent stannous fluoride) prior to topical application of 10 per cent aqueous solution of stannous fluoride would promote a significantly greater enamel fluoride uptake than when the solution was applied alone; and (2) to determine whether rinsing the mouth with tap water immediately after these procedures would significantly affect enamel fluoride uptake.

A modification of an *in vivo*, acid-etched enamel biopsy technique was used to measure fluoride and calcium contents of the outermost layer of enamel in first permanent molars and primary cuspids of 89 children. Four sites were biopsied prior to treatment. Treatments involved prophylaxes with a zirconium silicate paste either containing or not containing nine per cent stannous fluoride, followed by either a four-minute topical application of 10 per cent stannous fluoride in an aqueous solution or no such topical application. Some participants were instructed not to rinse for one-half hour; others were immediately offered unlimited quantities of tap water. Four other sites were biopsied one week after treatment; four more were biopsied two weeks after treatment.

Pre- versus post-treatment differences were measured via a fluoride-sensitive electrode and atomic absorption spectrophotometry.

Results were inconclusive. Enamel fluoride uptakes for most treatment groups appeared to be negative. Due to this seeming disagreement with the findings of many other investigators, and due to the very small numbers within treatment groups and biopsy sites, it was felt that uptake comparisons from group to group would be misleading. Tentative explanations for the unexpected results were offered.